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THE DIAGNOSTIC VALUE OF SOLUBLE TRANSFERRIN RECEPTOR IN HAEMODIALYSED PATIENTS

ZNACZENIE DIAGNOSTYCZNE ROZPUSZCZALNEGO RECEPTORA TRANSFERYNY U CHORYCH HEMODIALIZOWANYCH

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Summary

Introduction. Transferrin (Tf) donates iron into cells through its interactions with a membrane transferrin receptor (TfR) the quantity of which is proportional to the functional iron deficiency in the tissues. THE AIM OF THIS STUDY: Evaluation of the clinical usefulness of soluble transferrin receptor (sTfR), a truncated monomer of TfR, in patients treated with maintenance haemodialysis.
Materials and methods. Forty patients treated with maintenance haemodialysis (mHD) and 40 healthy volunteers (HV) were included to the study. In both studied groups serum sTfR concentration, blood cell count, selected iron metabolism indicators, serum parathormon (PTH) and sTfR index were assessed. Serum sTfR concentration was determined by immunonephelometry method. Baseline biochemical parameters (i.e. blood cell count, selected iron metabolism indicators) were measured by routine laboratory methods. Tf concentration was assessed by the rocket immunelectrophoresis according to Laurell. The sTfR index was calculated as a ratio of sTfR serum concentration to logarithm of serum ferritin concentration.
Results. All patients included to the study were anaemic. In mHD patients serum concentration of sTfR, iron and the value of the sTfR index were significantly lower as compared to HV. PTH serum concentration was significantly higher in mHD patients than in HV group. In mHD group statistically significant positive correlations between sTfR concentration and selected variables such as age, weight and body mass index (BMI) were observed. Moreover, it was found, among other, that PTH positively correlated with serum sTfR in mHD group.
Conclusions. sTfR serum concentration together with other parameters, i.e. serum concentrations of iron, transferrin and ferritin may be a useful indicator for iron status evaluation in patients treated with mHD. There are some parameters, like age, weight, body mass index (BMI) and PTH that may influence sTfR serum concentration.

KEY WORDS: soluble transferrin receptor, iron, anaemia, chronic renal failure, haemodialysis treatment.

Streszczenie

Wprowadzenie. Transferyna (Tf) dostarcza żelazo do wnętrza komórek poprzez interakcję z błonowym receptorem transferyny (TfR), którego ilość jest proporcjonalna do czynnościowego niedoboru żelaza w tkankach.
Cel pracy. Ocena klinicznej użyteczności rozpuszczalnego receptora transferyny (sTfR), monomeru TfR, w grupie chorych leczonych przewlekłą powtarzaną hemodializą.
Materiał i metody. Do badania włączono 40 chorych leczonych przewlekłą powtarzaną hemodializą (pHD) i 40 zdrowych ochotników (ZDR). W obu badanych grupach oceniono stężenie sTfR w surowicy krwi, morfologię krwi, wybrane parametry gospodarki żelazowej, stężenie parathormonu (PTH) oraz wskaźnik sTfR. Stężenie sTfR badano metodą immunonefelometryczną. Podstawowe parametry biochemiczne (morfologia krwi i wybrane wskaźniki gospodarki żelazowej) były oceniane rutynowymi metodami laboratoryjnymi. Stężenie Tf badano metodą immunoelektroforezy rakietkowej wg Laurella. Wskaźnik sTfR został wyliczony na podstawie wzoru: stężenie sTfR w surowicy krwi/logarytm ze stężenia ferrytyny w surowicy krwi.
Wyniki. Wszyscy chorzy włączeni do badania wykazywali cechy niedokrwistości. Stężenie sTfR, stężenie żelaza oraz wartość wskaźnika sTfR były istotnie obniżone u tych chorych w porównaniu ze ZDR. Stężenie PTH było istotnie statystycznie wyższe w grupie pHD w stosunku do grupy ZDR. W grupie pHD zaobserwowano występowanie istotnych statystycznie zależności pomiędzy stężeniem sTfR a wybranymi zmiennymi, takimi jak: wiek, masa ciała i wskaźnik masy ciała (BMI). Ponadto wykryto m.in. istnienie wprost proporcjonalnych zależności pomiędzy PTH a stężeniem sTfR w grupie pHD.
Wnioski. Stężenie sTfR w surowicy krwi razem z innymi wskaźnikami, tj. stężeniem żelaza w surowicy krwi oraz stężeniem ferrytyny może być użytecznym parametrem w ocenie stanu gospodarki żelazowej u chorych leczonych pHD. Istnieją czynniki, takie jak: wiek, masa ciała, BMI i PTH, które mogą wpływać na stężenie sTfR w surowicy krwi.

SŁOWA KLUCZOWE: rozpuszczalny receptor transferyny, żelazo, niedokrwistość, przewlekła niewydolność nerek, leczenie hemodializą.

Introduction

Pathogenesis of anaemia associated with chronic kidney disease (CKD) is multi-factorial. Progression of CKD is associated with decreased erythropoietin production, inadequate iron availability and chronic inflammatory processes resulting from the disease per se and from one of the

ways of treatment i.e. treatment with maintenance haemodialysis (mHD) [1].

Therefore, the essential quality of appropriate treatment of anaemia in CKD is the evaluation of the body iron status [2, 3].

Unfortunately, the laboratory tests widely used in clinical practice, i.e. serum iron, total iron-binding capacity (TIBC), transferrin saturation (TSAT) and serum ferritin (sFn) are considerably influenced by a number of non-related conditions, e.g. acute phase reactions, which may complicate the clinical interpretation of their results and create inaccurate picture of the body iron status.

So currently, there is still no single biochemical or haematological parameter that is sensitive or specific enough to completely describe the distribution of iron in the human body. One of the suggested parameters is soluble transferrin receptor (sTfR) [4, 5]. The evaluation of this parameter is an indirect measure of membrane transferrin receptor quantity. This assumption is based principally on the observed correlation between concentration of sTfR molecules complexes with transferrin (Tf) and ferrokinetic measurements of erythron Tf uptake [6, 7].

Aim of the study

This study was undertaken to evaluate the clinical usefulness of sTfR in iron status assessment in haemodialysed patients, supplemented intravenously with iron.

Materials and Methods

This study was carried out on a group of 80 subjects: 40 patients treated with maintenance haemodialysis (mHD group) and 40 healthy volunteers (HV) as controls.

Ethical approval was obtained from the Local Ethics Committee before the start of the study and all patients were asked for their verbal consent before the blood was taken.

Patients with acute inflammatory processes and malignant tumours were excluded from the study.

Haemodialysis procedures were performed three times a week for four hours with polysulphone capillary dialysers. After HD, depending on the concentrations of selected haematological variables, patients have been supplemented intravenously with erythropoietin alpha (mean weekly doses 4300 ± 350 IU) and iron sucrose (mean weekly doses 112 ± 5 mg).

Blood samples were drawn from mHD patients at the start of HD, before the second dialysis session of the week at the same time as their usual monitoring blood tests. Reference values were obtained in the morning, from fasting HV, with negative history of an acute or chronic inflammation and without any laboratory and clinical symptoms of anaemia.

The RBC [10^{12}/l], HCT [%], HGB [g/dl], mean corpuscular haemoglobin (MCH) [pg], mean corpuscular volume (MCV) [fl] were estimated by a conductometry method, using haematological analyser (Sysmex K-4500, ICN).

Serum iron concentration, the total iron binding capacity (TIBC) [µmol/l] and unsaturated iron binding capacity (UIBC) [µmol/l] levels and [µmol/l] were evaluated by a colorimetric method with ferrozin, using biochemical analyser (Cobas Integra, Roche).

sFn [ng/ml] was estimated by a chemiluminescence's method with mice monoclonal antibodies specific to human ferritin, using immunochemical analyser (Elecsys 2010, Roche Diagnostic Ltd).

TSAT [%] was calculated as a ratio of serum iron concentration to TIBC value.

Serum concentration of sTfR [mg/l] was determined by an immunonephelometry method, using N Latex sTfR (DADE Behring) and nephelometer (DADE Behring Analyzer II). Polystyrene particles coated with monoclonal antibody specific to human sTfR were aggregated when mixed with samples containing sTfR. These aggregates scattered a beam of light passed through the sample. The intensity of the scattered light was proportional to the concentration of relevant protein in the sample. The results were evaluated by comparison with a standard of known concentration.

The sTfR index was assessed as a ratio of sTfR concentration to logarithm of serum ferritin concentration.

Table 1. General characteristics of the studied subjects, i.e. patients treated with maintenance haemodialysis (mHD) and healthy volunteers (HV)
The values are means ± SD

Studied groups	Age [year]	Weight [kg]	BMI [kg/m^2]	Diagnosis			Duration of ESRD before enrolling to HD [months]	Duration of HD [months]
				GN	DN	HN		
mHD n = 40	52.6 ± 14	73.75 ± 14.6	24.3 ± 4.8	10	12	18	52 ± 2	46 ± 6
HV n = 40	42.1 ± 5	65.75 ± 10.5	22.6 ± 4.3	----------				

n – number of studied subjects, BMI – body mass index, ESRD – end-stage renal disease
GN – glomerulonephritis, DN – diabetic nephropathy, HN – hypertensive nephrosclerosis
HD – haemodialysis treatment

To measure Tf [mg/l] concentration the rocket immunelectrophoresis according to Laurell [8] with rabbit human anti-Tf (DAKTOPATTS) was used.

Parathormon (PTH) [pg/ml] serum concentration was measured by an immunometric assay based on monoclonal antibodies using the Elecsys PTH Immunoassay (Elecsys 1010 System, Roche Diagnostic Ltd).

All tests were performed according to the manufacture's instruction.

BMI – body mass index was assessed as a ratio of body mass [kg] to height [m^2].

Statistical analysis was performed using the computer software Statistical Package (*STATISTICA 6.0.*). Data were expressed as mean values ± SD (standard deviation) for 40 subjects in each group. For the analysis of the normally distributed variables Shapiro-Wilk test was used. Non-parametrical tests were performed for the analysis of the non-normally distributed variables. For comparison of two groups, unpaired Student's t - test was used for normally distributed variables and Mann–Whitney rank sum test for variables with non-normal distribution. Unvaried correlations were performed using Spearman rank test. Pearson's χ^2 test and linear regression coefficients were used for frequency measures. The differences were considered significant at $p < 0.05$.

Results

All mHD patients included to the study were anaemic at the time of blood sampling: their values of RBC, HGB, HCT, TIBC, UIBC and serum iron were significantly lower while TSAT and sFn values were significantly higher in comparison to HV.

Serum concentration of sTfR was significantly lower ($p = 0.01$) in mHD patients as compared to HV (Table 2).

PTH serum concentration was significantly higher ($p < 0.00001$) in mHD patients (1028.8 ± 1202.4 pg/ml) than in HV group (32.2 ± 25.5 pg/ml).

Statistically significant positive correlations between sTfR concentration and selected variables such as age, weight and BMI were observed in mHD group (Table 3).

Moreover, RBC, Tf, TIBC, UIBC and PTH positively correlated with serum sTfR and the significant inverse correlations were observed between sTfR and Fe, sFn, TSAT and MCH values.

Positive correlations, which did not achieve statistical significance, were observed between sTfR concentration and HGB and HCT (Table 4).

The assessment of iron status was also obtained from the sTfR index [sTfR concentration/log of serum ferritin concentration]. The value of this index was statistically lower ($p < 0.05$) in mHD group (0.15 ± 0.1) than in HV (0.96 ± 0.58).

Strong negative correlation between the value of sTfR index and serum Fe concentration was revealed. Positive correlation between this index and Tf serum concentration was also found. However, there were no correlations between the sTfR index and selected serum variables such as HGB and HCT (Table 5).

Table 2. The concentrations of selected haematological variables in studied subjects, i.e. patients treated with maintenance haemodialysis (mHD) and healthy volunteers (HV)

variable	mean value ± SD		p
	mHD	HV	
RBC [10^{12}/l]	3.21 ± 0.51	4.63 ± 0.83	< 0.00001
HCT [%]	33.27 ± 3.16	44.26 ± 7.2	< 0.00001
HGB [g/dl]	10.68 ± 1.57	14.95 ± 2.03	< 0.00001
MCH [pg]	33.13 ± 5.22	29.30 ± 3.30	< 0.00001
MCV [fl]	98.74 ± 5.97	89.52 ± 8.58	< 0.00001
TIBC [µmol/l]	41.84 ± 8.34	57.98 ± 13.38	< 0.00001
UIBC [µmol/l]	17.14 ± 9.18	39.25 ± 3.57	< 0.00001
Fe [µmol/l]	14.40 ± 7.51	18.73 ± 9.09	0.01
sFn [ng/ml]	1608.13 ± 168.2	188.23 ± 95.32	< 0.00001
Tf [mg/l]	1456.12 ± 464.94	3525.11 ± 725.22	< 0.00001
sTfR [mg/l]	0.85 ± 0.5	1.4 ± 0.2	0.01
TSAT [%]	36.68 ± 22.67	32.5 ± 6.5	0.21

Table 3. The correlations between serum sTfR concentration and selected anthropometric variables in patients treated with maintenance haemodialysis (mHD)

pairs of variables	r	p
sTfR and age	0.33	0.02
sTfR and weight	0.43	0.0025
sTfR and BMI	0.37	0.01

BMI – body mass index (kg/m^2)

Table 4. The correlations between serum concentration of sTfR and selected blood cell indices, iron metabolism variables and parathormon serum concentration in patients treated with maintenance haemodialysis (mHD)

pairs of variables	r	p
sTfR and RBC	0.29	0.04
sTfR and HGB	0.25	0.07
sTfR and HCT	0.24	0.08
sTfR and MCH	-0.34	0.02
sTfR and TIBC	0.34	0.02
sTfR and UIBC	0.36	0.02
sTfR and Fe	-0.34	0.03
sTfR and sFn	-0.29	0.04
sTfR and Tf	0.30	0.03
sTfR and TSAT	-0.29	0.04
sTfR and PTH	0.37	0.025

Table 5. The correlations between sTfR index values and selected haematologic variables in mHD group

pairs of variables	r	p
sTfR index and Fe	-0.53	0.0003
sTfR index and Tf	0.30	0.03
sTfR index and HGB	-0.12	0.43
sTfR index and HCT	-0.06	0.69

Discussion

Recently, serum sTfR [9], a truncated monomer of tissues receptor arising as a result of its proteolysis, has been proposed as a tool for improving the evaluation of iron availability for erythropoiesis in patients with ESRD, particularly when "functional iron deficiency" is due to chronic disorders.

It is known that if the iron supply is inadequate there is an up-regulation of sTfR synthesis to enable the cell to compete more effectively for iron. An increase in sTfR concentration is observed in patients with iron deficiency anaemia, regardless of whether there is an inflammation [10]. However, if sTfR serum concentration is in a normal range, anaemia of chronic disorders should be taken into account. On this basis it is proposed that the estimation of sTfR serum concentration may be useful for differentiating iron deficiency anaemia from anaemia of chronic disorders. The problem occurs if anaemia of chronic disorder coexists with iron deficiency anaemia. In this case serum sTfR concentration is increased and without any information about other parameters, such as serum Tf concentration among other, it is very difficult to separate these two processes. In this case, the decreased or normal Tf concentration may suggest anaemia of chronic disorders, and the increased Tf level may show that these two types of anaemia coexist [11, 12].

In haemodialysed patients we have observed a lower serum concentration of iron and sTfR and a higher concentration of transferrin and serum ferritin as compared to HV. This lower sTfR concentration may suggest, that there are some other unknown factors, apart from body iron status, that may have an influence on the serum concentration of sTfR.

Until now, it was suggested that sTfR concentration is marked by a little individual changeability, but we have found that serum sTfR concentration in uraemic patients correlates positively with age, weight and BMI. We have also revealed that serum concentration of parathormon correlates positively with serum concentration of sTfR. This relationship between PTH and sTfR serum concentration was also presented in the publication concerning renal transplant patients and patients treated with renal replacement therapy [13].

So, we have suggested that age, weight, BMI and PTH, may have an influence on sTfR.

In our study, we have confirmed, that sTfR concentration correlates positively with RBC, Tf, TIBC and negatively with MCH. The similar correlations have been observed by the American investigators in case of the patients undergoing evaluation of iron stores [14] and by the British scientists in case of the patients with rheumatic arthritis [15].

Our results have shown that sTfR serum concentration, if taken into account together with other parameters, such as Tf, sFn and iron concentration may be useful as a test for assessment of the iron metabolism state in patients treated with mHD.

For detecting latent iron deficiency in studied patients [16, 17, 18] we have calculated sTfR index (the ratio of sTfR to serum ferritin using logarithmic transformation). The mean value of this index was significantly lower in haemodialysed patients if compared to healthy volunteers. The increased serum ferritin concentration observed in studied patients influences sTfR index value. In these conditions we have suggested that sTfR index in body iron status assessment in haemodialysed patients has questionable value.

Conclusions

1. The sTfR serum concentration, taken into account together with other parameters, such as serum iron concentration, transferrin and ferritin may be useful as a test for evaluation of iron state in patients treated with maintenance haemodialysis.

2. There are some parameters, like age, weight, BMI and PTH which may influence sTfR serum concentration.

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